AD-A259 830

(2)

OFFICE OF NAVAL RESEARCH

Grant N00014-92-J1052

R&T Code 4135018

Technical Report #6

"Specific Recognition of CG Base Pairs by 2-Deoxynebularine within the Purine•Purine•Pyrimidine Triple-Helix Motif"

by
H. U. Stilz and P. B. Dervan

California Institute of Technology
Division of Chemistry and Chemical Engineering
Pasadena, CA

January 11, 1993

\\004

93 ... 8

Specific Recognition of CG Base Pairs by 2-Deoxynebularine within the Purine•Purine•Pyrimidine Triple-Helix Motif+

Hans Ulrich Stilz and Peter B. Dervan*

Beckman Institute

California Institute of Technology

Pasadena, California 91125

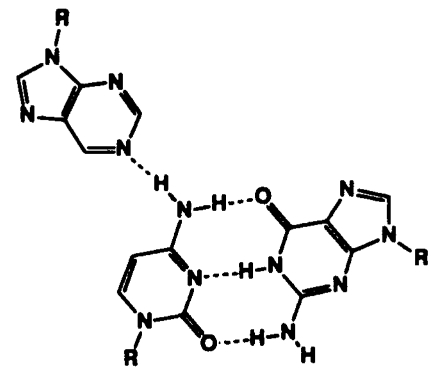
RUNNING TITLE: Recognition of CG-base pairs

+ We are grateful to the Office of Naval Research for generous support

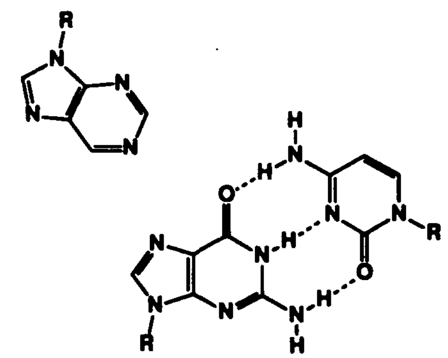
* To whom correspondence should be addressed

ABSTRACT

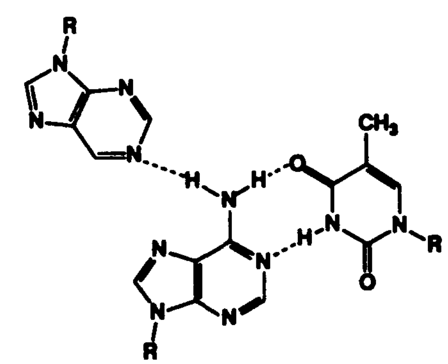
The sequence-specific recognition of double-helical DNA by purine-rich oligodeoxyribonucleotide-directed triple-helix formation is limited to purine tracts. Within the geometric constraints of the phosphate-deoxyribose position of a purine•purine•pyrimidine triple helical structure model building studies suggested that the deoxyribonucleoside 2'-deoxynebularine (**dN**) might form one specific hydrogen bond with cytosine (C) or adenine (A) of Watson-Crick cytosine-guanine (CG) or adenine-thymine (AT) base pairs. 2-Deoxynebularine (**dN**) was incorporated by automated methods into purine-rich oligodeoxyribonucleotides. From affinity cleavage analysis, the stabilities of base triplets within a purine•purine•pyrimidine (pu•pu•py) triple helix were found to decrease in order N•CG~N•AT >>N•GC~N•TA (pH 7.4, 37 °C). Oligodeoxyribonucleotides containing two N residues were shown to bind specifically within plasmid DNA a single 15 base pair site of the human immunodeficiency virus genome containing two CG base pairs within a purine tract. This binding event occurs under physiologically relevant pH and temperature (pH 7.4, 37 °C) and demonstrates the utility of the new base. Quantitative affinity cleavage titration reveals that, in the particular sequence studied, an N•CG base triplet interaction results in a stabilization of the local triple helical structure by 1 kcal•mol^{-1} (10 mM NaCl, 1 mM spermine tetrahydrochloride, 50 mM Tris-acetate, pH 7.4, 4 °C) compared to an A•CG base triplet mismatch.



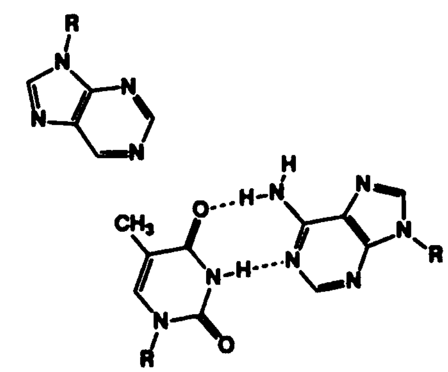
N•CG



N•GC



N•AT



N•TA

Figure 1

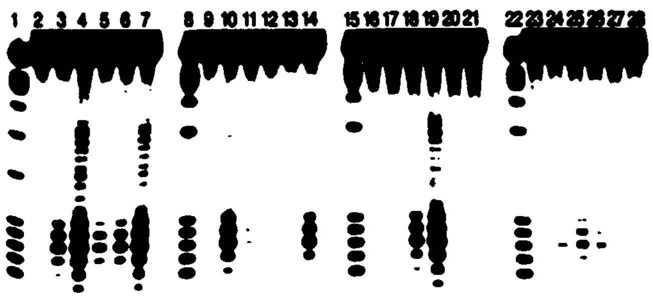
Oligo	Z
1	N
2	A
3	G
4	C
5	T

X = A G C T
Y = T, C, G, A

^{32}P 5'-AATTCTCTCTAAAAAGGGXGGGGAGGGGAGGGAAAAACTCTCT
GAGAGATTTTTCCCYCCCCTCCCCTCCCTTTTTGAGAGAGATC-5'

3'-T*GGGZGGGGTGGGGT-5'

Figure 2A


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28

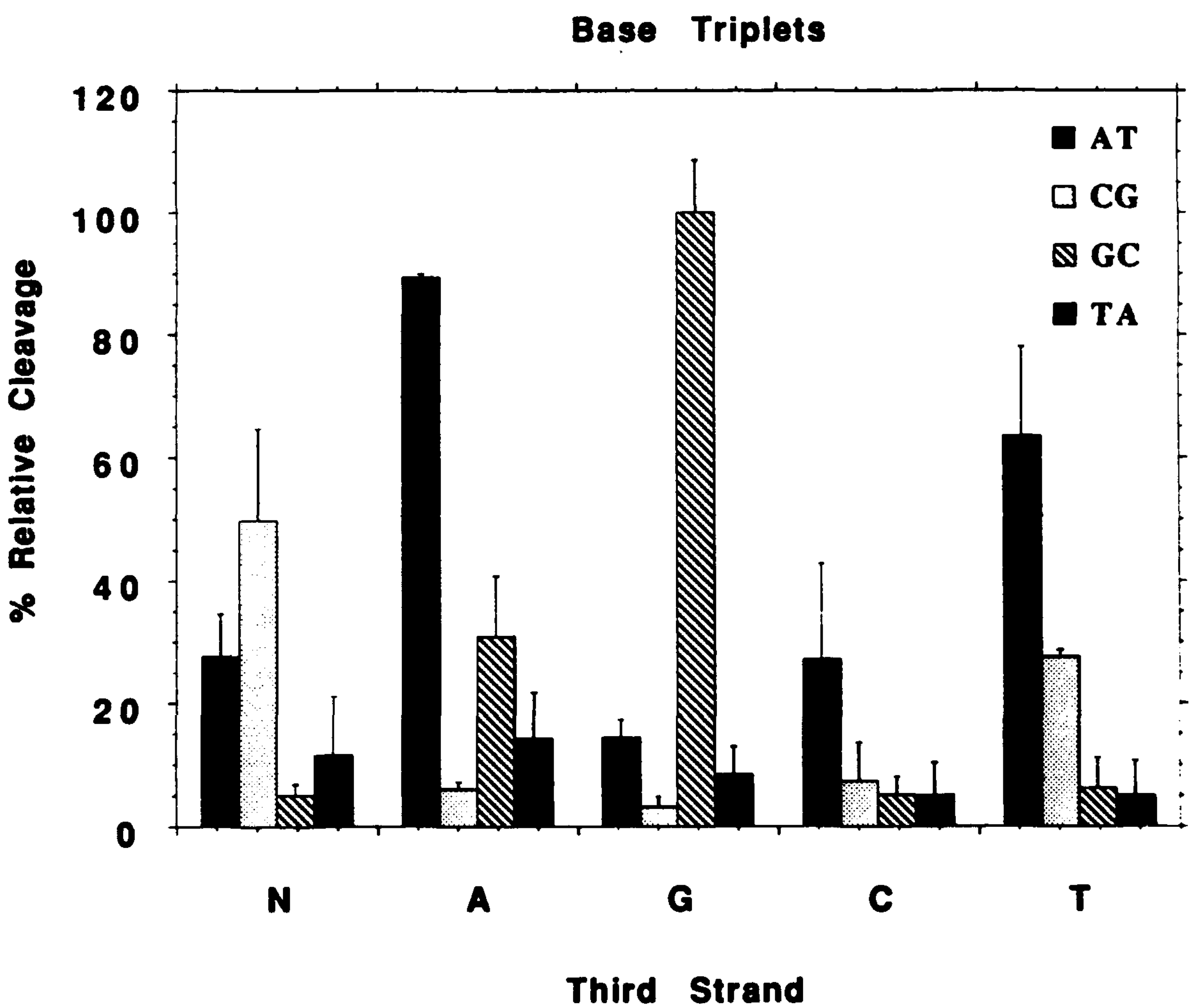
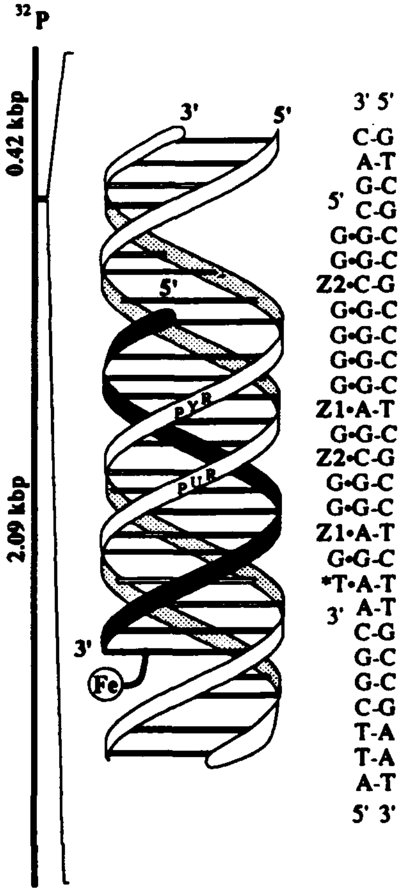
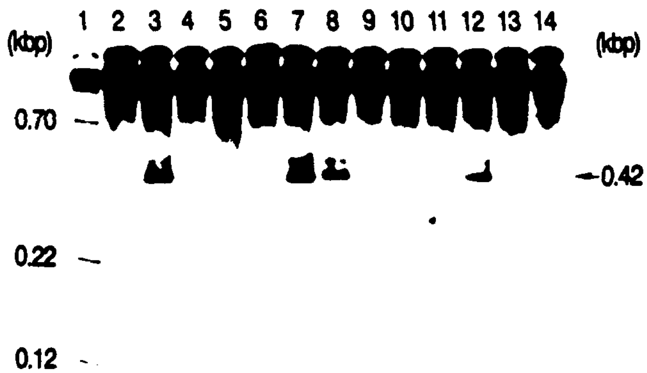

Base Triplets
120
100
80
60
40
20
0
% Relative Cleavage
AT
CG
GC
TA
N
A
G
C
T
Third Strand

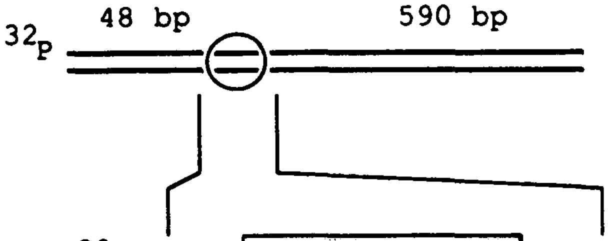
Figure 3



Oligo	Z1	Z2
6	A	N
7	A	A
8	A	G
9	A	C
10	A	T
11	T	N
12	T	A
13	T	G
14	T	C
15	T	T
16	N	N
17	N	T

Figure 4A

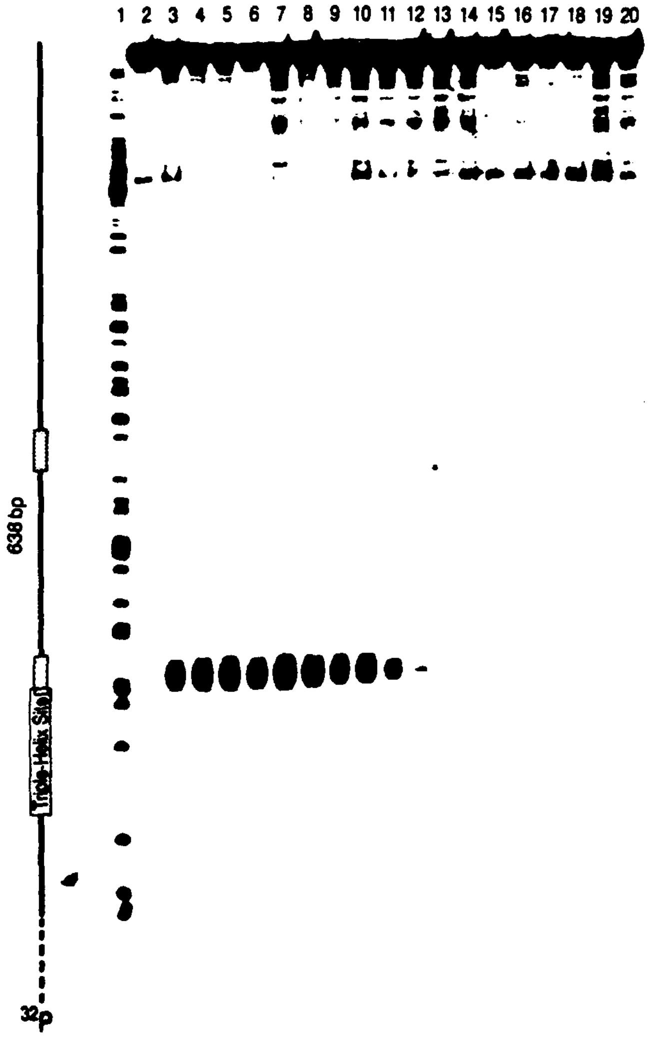

1 2 3 4 5 6 7 8 9 10 11 12 13 14
(kbp)
(kbp)
0.70
0.42
0.22
0.12

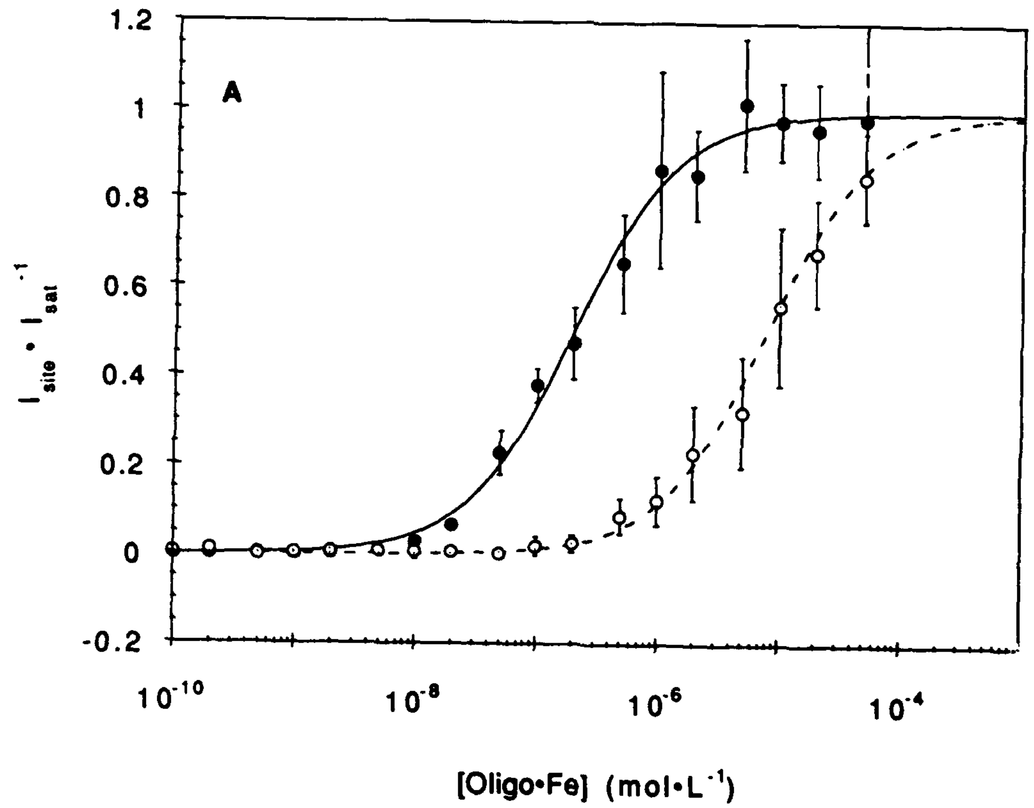


3'-^{32}P·· CAGCGGCGGGGAGCGGAGAACGG-5'
5'- GTCGCCGCCCCTCGCCTCTTGCC-3'

6 5' - GG**N**GGGG**A**G**N**GG**A**GT*
7 5' - GG**A**GGGG**A**G**A**GG**A**GT*
10 5' - GG**T**GGGG**A**G**T**GG**A**GT*
11 5' - GG**N**GGGG**T**G**N**GG**T**GT*
17 5' - GG**T**GGGG**N**G**T**GG**N**GT*

Figure 5


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
638 bp
Triple-Helix Site
32P



A
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
$I_{site} \cdot I_{sat}^{-1}$
10^{-10}
10^{-8}
10^{-6}
10^{-4}
[Oligo•Fe] (mol•L^{-1})

Figure 7A

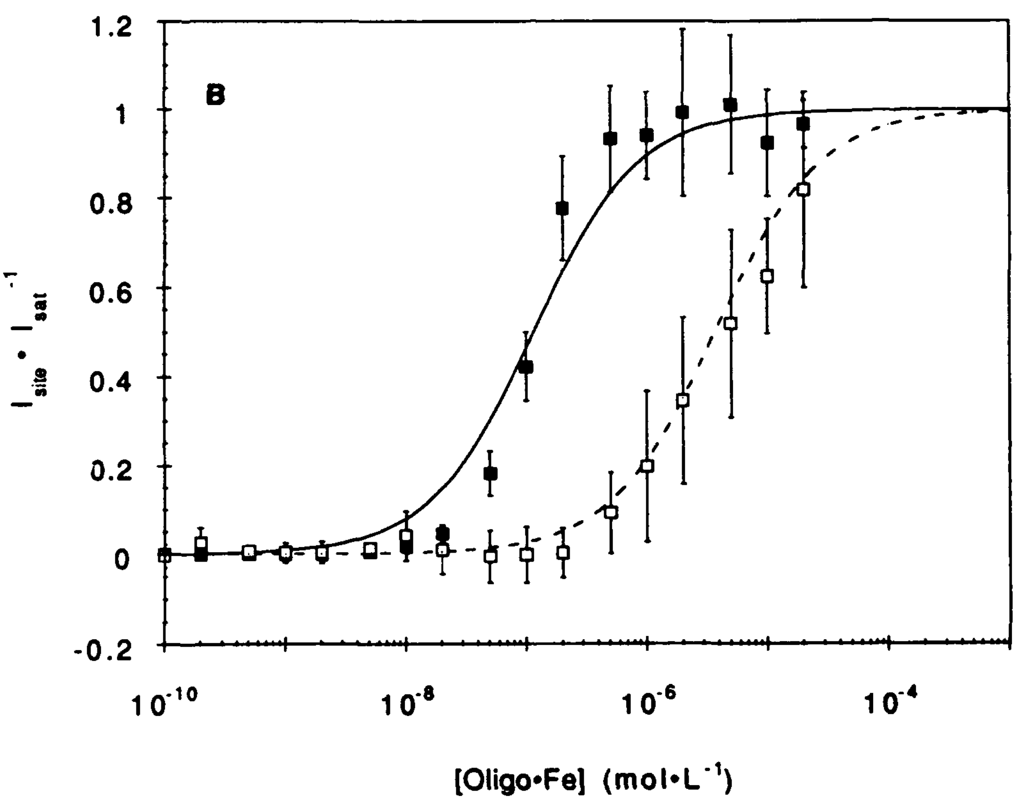

B
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
$I_{site} \cdot I_{sat}^{-1}$
10^{-10}
10^{-8}
10^{-6}
10^{-4}
[Oligo•Fe] (mol•L^{-1})

Figure 7B

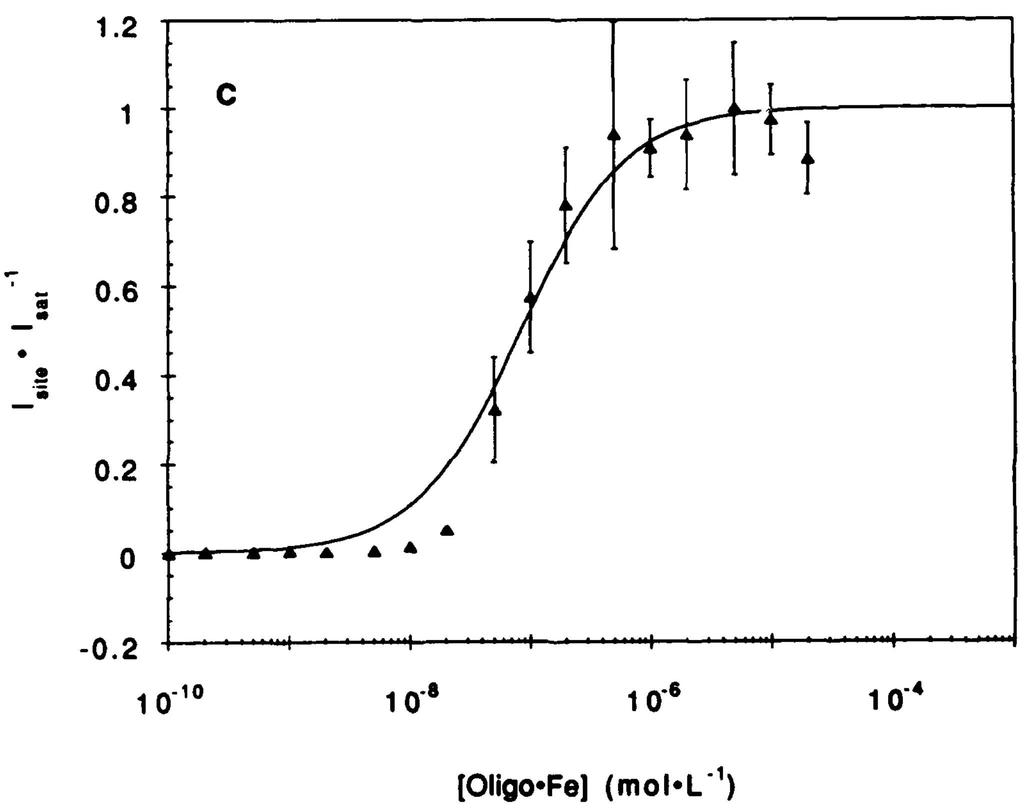
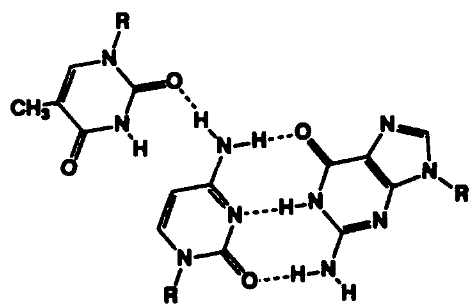
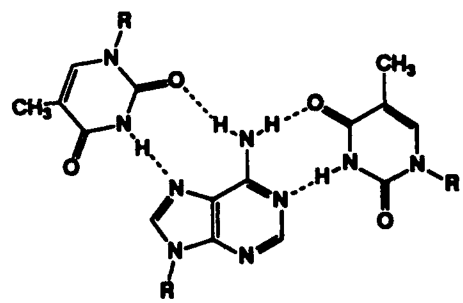

C
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
$I_{site} \cdot I_{sat}^{-1}$
10^{-10}
10^{-8}
10^{-6}
10^{-4}
[Oligo•Fe] (mol•L^{-1})

Figure 7 C



T•CG



T•AT

Figure 8

Table I: Association Constants at pH = 7.4 and 4 ^oC.[a]

Oligo	Base Triplets (n)[b]	K_T (M^{-1})	ΔG_T (kcal•mol^{-1})
6	G•GC (10), A•AT (2), N•CG (2)	5.0 (± 1.8) x 10^7	- 9.8 (± 0.3)
7	G•GC (10), A•AT (2), A•CG (2)	1.3 (± 0.5) x 10^6	- 7.8 (± 0.3)
10	G•GC (10), A•AT (2), T•CG (2)	1.2 (± 0.3) x 10^8	- 10.2 (± 0.3)
11	G•GC (10), T•AT (2), N•CG (2)	8.6 (± 2.0) x 10^7	- 10.1 (± 0.2)
17	G•GC (10), N•AT (2), T•CG (2)	2.7 (± 2.0) x 10^6	- 8.2 (± 0.8)

[a]Values listed are mean values measured from affinity cleavage titration experiments performed in Association buffer (10 mM NaCl, 1 mM spermine tetrahydrochloride, 0.1 mM-bp calf thymus DNA, 50 mM Tris-acetate, pH 7.4).
[b] n = number of triplets within each triple helical complex.